Ex-3

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 406

THE ROLE OF ANIMALS IN BIOMEDICAL RESEARCH

Edited by Jeri A. Sechzer

The New York Academy of Sciences
New York, New York
1983

Library of Congress Cataloging in Publication Data
Main entry under title:

The Role of animals in biomedical research.

(Annals of the New York Academy of Sciences ; v. 406)
Bibliography: p.
Includes index.
1. Animal models in research. I. Sechzer, Jeri A., 1930- . II. Series. [DNLM: 1. Animals, Laboratory—Congresses. 2. Research—Methods—Congresses. WI AN626YL v. 406 / QY 58 R745 1982]
Q11.N5 vol. 406 [QL55] 500s [619] 83-13047
ISBN 0-89766-204-0
ISBN 0-89766-205-9 (pbk.)

PCP
Printed in the United States of America
ISBN 0-89766-204-0 (cloth)
ISBN 0-89766-205-9 (paper)

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 406

June 20, 1983

THE ROLE OF ANIMALS IN BIOMEDICAL RESEARCH*

Editor and Conference Chairman

JERI A. SECHZER

CONTENTS

* This volume is the result of a workshop entitled The Role of Animals in Biomedical Research held on April 28–29, 1982 by the New York Academy of Sciences.

THE USE OF SHORT TERM *IN VITRO* AND SUBMAMMALIAN TESTS AS ALTERNATIVES TO LARGE SCALE ANIMAL BIOASSAYS

David J. Brusick

Department of Molecular Toxicology
Litton Bionetics, Inc.
Kensington, Maryland 20895

INTRODUCTION

Safety testing and related toxicology research represent one of the largest uses of laboratory research animals.

From the point of view of the chemical and pharmaceutical industries there is clearly an interest in and a recognized need for molecular toxicology studies, which serve as alternatives to the dependency on large scale animal studies. This need appears to be based more upon the ability to predict toxic hazards in a short time and the favorable economics associated with safety testing programs employing *in vitro* testing and not to a great extent on moral or ethical issues of using animals as test organisms.

Summarized below are some of the factors that are at work in the field of safety testing that are moving much, but not all, of the testing from intact mammals to mammalian *in vitro* and submammalian model systems.

The most widespread application involves the use of *in vitro* tests to identify presumptive carcinogens. *In vivo* life-time studies in rodents or other animal species are presently the only methods for carcinogen assessment that are recognized in making regulatory decisions. However, the cost (presently $600,000–$1,000,000 per chemical) and performance time (two to three years) for the rodent bioassay are of sufficient magnitude to warrant preliminary testing with *in vitro* predictive tests in order to assist in the decision to invest corporate resources in these more expensive toxicology analyses. Using *in vitro* systems, which are highly reliable and can be quite predictive, tests on candidate compounds can be performed within three months for approximately 1/10 or 1/20 the cost of a single rodent cancer study.

Many industrial chemicals that do not require carcinogen testing are nevertheless involved in significant human exposures, which would justify having some information that estimates the carcinogenic potential of these materials in order to protect production workers and endproduct consumers. Short-term *in vitro* tests are often the only source of this safety information.

Cancer is fundamentally a cellular process that arises from specific alterations in the control mechanisms of individual cells. It is often difficult to establish a mechanism of action for agents that increases the tumor incidence in a rodent species under animal bioassay conditions. One of the advantages of short-term and *in vitro* techniques is their intrinsic potential to study the mechanisms of neoplasia at the cellular and molecular levels.

Occasionally, unexpected sex, species, or strain-specific responses are encountered that might be resolved if the tumorigenic mechanism of the material were understood. *In vitro* techniques have been used to resolve problems involving differential responses in target strains, species, and organs. Short-term tests

0077-8923/83/0406–0068 $01.75/2

may be useful in resolving the initiating, promoting, or co-carcinogenic properties of a test material—something not readily obtained from results of the animal tests.

Thus, from these examples, it should be evident that nonanimal model systems can and will play an increasingly important role in chemical safety testing, but the forcing factors will likely be based on scientific and economic issues.

Rationale for Conducting Mutagenesis Testing

Before an industrial compound can be marketed, information must be generated concerning the toxic effects obtained in animals exposed to the particular compound under a variety of conditions; information gained from this type of testing is then extrapolated to anticipated human exposure. At a minimum, the information includes results involving acute toxicity. Increasingly, premarketing information is also focusing on teratogenicity (the development of birth defects) and carcinogenicity (the development of cancer). Testing for these endpoints normally requires substantial *in vivo* mammalian assays. Recently, *in vitro* mutagenicity testing has been recommended as a possible alternative for *in vivo* models under certain circumstances. Testing for mutagenicity *per se* is not yet a premarketing requirement for new consumer products, although it may be in the near future.

Mutagen Detection

The integrity of the human gene pool is critical to the health status of the species and must be protected from exposure to chemicals capable of inducing mutagenic changes.[4] The consequences of mutation induction depend on the cells that carry the mutation (FIGURE 1). Mutation may occur in two different types of cells, somatic or germinal. Mutations in germinal cells may be transmissible genetic alterations, which can affect subsequent generations.[6] Expression of the germ cell mutation may be immediate in the following generation or it may be expressed many generations in the future.

The bulk of the cells that make up an individual are not germinal cells but are somatic cells. These include all the cells that are not part of the reproductive system. Genetic damage in somatic cells has been associated with the production of neoplasia and teratogenicity in animals.[8] Other diseases, such as those associated with aging, have also been attributed to mutation or unrepaired DNA damage.[8]

Carcinogen Detection

The genetic basis for malignancy has been fairly well demonstrated through a number of studies that have reported chromosome aberrations and/or altered gene activity in tumor cells, and by the good correlation between chemicals that are carcinogens and also exhibit mutagenic activity.[1,3] At least 95 percent of all chemicals that can initiate cancer in animals will also produce mutations in one or more types of *in vitro* genetic screening systems, and conversely chemicals that show positive effects in mutagenicity assays have an 80 percent probability that they will also be carcinogenic in rodents.

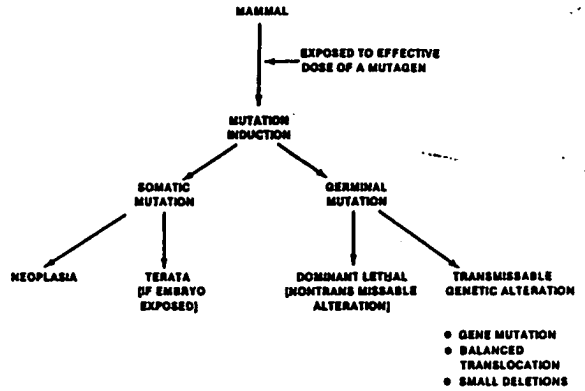

FIGURE 1. Possible consequences of mutation induction in somatic and germ cells of mammals. (From D. J. Brusick.[9] With permission from Elsevier/North-Holland Biomedical Press.)

Teratogen Detection

Exposure of pregnant females to a chemical mutagen might place a critical stem cell in the developing embryo at risk. These cells normally give rise to a large number of additional cells, producing either an organ system or a structural component in the developing embryo. If a mutation affects a precursor or a stem cell, it may generate defective cells unable to differentiate, resulting in a terata, or a deformed embryo. *In vitro* tests for mutation induction or chromosome damage may be useful in detecting potential teratogenic agents or chemicals that may affect fertility by altering DNA replication or transciption.

Thus, in addition to the identification of a chemical that presents a genetic hazard to humans, *in vitro* or small-scale *in vivo* genetic assays can provide information of a more immediate usefulness, the prediction of chemicals that can possibly cause toxicity directly to the affected individual.

Development of Nonanimal Model Systems

Not all types of toxicity tests are amenable to *in vitro* or submammalian alternative techniques. The three types of toxic phenomena discussed previously, genetic disease, cancer, and terata, however, are uniquely adaptable. They all require relatively large animal studies, the effects observed occur at dose levels that are not acutely toxic to the target species, and they have an underlying commonality in the mechanisms(s) of induction. All three of these phenomena are the product directly or secondarily of altered gene function.[1,2,9] Thus, by assessing chemicals for their genetic or genotoxic properties, one can develop judgements

about the potential of these agents as mutagens, carcinogens, and possibly teratogens.

In establishing nonanimal alternatives, two important criteria must be met: (1) The alternative test, if implemented on a routine basis, will not result in a health risk to humans greater than that presently permitted by use of the animal model (i.e., the number of false negative responses in the alternative should be no greater than the animal test it is replacing). (2) The introduction of the alternative test will lead to greater efficiency in the assessment of the particular toxic endpoint(s) than currently available animal models.

In the case of genetic testing, these two criteria appear to be attainable. For example, TABLE 1 compares the test performance of short-term tests for genotoxicity with the standard rodent bioassay for identifying human carcinogens. The results show that there is approximate equality between the two tests in making an accurate designation. Neither test is a perfect model but there appears to be no loss in the ability to protect humans from carcinogens when nonanimal techniques are used.

TABLE 1
A COMPARISON OF SHORT TERM TEST RESULTS AND ANIMAL BIOASSAY RESULTS AS PREDICTORS OF HUMAN CARCINOGENS

Chemical	IARC Status*	Short Term Test Results	Rodent Bioassay Results
4-aminobiphenyl	HC	+	+
Arsenic	HC	+	–
Asbestos	HC	Limited†	–
Benzene	HC	+	+
Benzidine	HC	+	+
BCME	HC	+	+
Chromium	HC	Limited	+
DES	HC	+	+
Melphalan	HC	+	+
Mustard gas	HC	+	+
2-Naphthylamine	HC	+	+
Vinyl chloride	HC	+	+
Aflatoxin	PHC	+	+
Dimethylsulfate	PHC	+	+
Cadmium	PHC	Limited	+
Chlorambucil	PHC	+	Limited
Acrylonitrile	PHC	+	+
Amitrole	PHC	Limited	+
Auramine	PHC	+	Limited
Beryllium	PHC	+	+
Carbon tetrachloride	PHC	–	+
Cyclophosphamide	PHC	+	+
Dimethylcarbamoyl chloride	PHC	+	+
Ethylene oxide	PHC	+	Limited

*IARC Monographs and Supplements through 1981.
†Limited = positive results reported but the entire data base is not unequivocal.
HC = human carcinogen and PHC = probable human carcinogen.

TABLE 2
COMPARISON OF CARCINOGEN ANALYSES WITH MOLECULAR AND RODENT BIOASSAYS

Parameter	Molecular Toxicology Approach	*In Vivo* Rodent Studies For Tumor Induction
Cost/analysis	$25,000	$600,000
Time required to complete	3 months	3 years
Number of animals employed	Liver from one animal	600
Space required per analysis	< 500 sq. ft. × 1 month	250 sq. ft. × 2.5 years

TABLE 2 provides an illustration of how short-term *in vitro* tests meet the second criterion listed previously. The efficiency of testing for carcinogens, when considering cost, performance time, and space utilized, increases by a factor of 10–20 when *in vitro* or submammalian models are used.

Other advantages of short-term tests could be listed, such as the ability to perform repeat confirmation testing on a routine basis and the opportunity to formulate predictive positions regarding three different toxic endpoints (genetic effects, cancer, teratology) on the results of a single type of test method.

Chemicals that do not have genotoxic activity in genetic screening tests can be said to have none of the biological properties associated with known rodent carcinogens or mutagens. That kind of information is useful, especially very early in the development of compounds destined for the market. If one can direct developmental resources for the compounds that have a high probability of reaching the market place and eliminate, very early, those that have a high probability of showing adverse toxicological effects, overall developmental resources will be conserved.[7]

Deployment of Mutagenesis Tests

There are different approaches to the use of these tests. One is to use a type of tier system in which one starts with the very simple test to screen large numbers of compounds and identify those that should be eliminated, the remaining compounds are subjected to further testing.

By going through the various levels of testing, one can obtain a profile of information on either positive compounds or negative compounds. If a decision is made to discard positive compounds at the very first level of testing, negative compounds can be followed through to make sure that they are going to continue to be free of any toxicological activity, regardless of the assay system used. Alternatively, one can choose to follow the positive compounds through a particular series of tests if they have potential economic importance, to determine if they are mutagenic or genetically active in more than a single test system. If the positives are followed through and become negative at higher phylogenetic levels, their potential for toxicity must be considered suspect. If, however, they continue to show up positive, then it is very unlikely that one would be able to clear this compound in full-scale standard animal toxicological evaluations before putting it on the market. Using a battery of tests conducted simultaneously is a more common application of short-term tests. In the battery approach, the activity profile of the test chemical can be developed most rapidly and accurately.[8]

In summary, then, these types of tests are useful for more than just measuring the mutagenicity of a compound per se. They are important in decisions on utilization of resources for other types of toxicological tests that require a major investment. These tests are also capable of determining the potential hazards of compounds that are going to the marketplace, but are not required to be subjected to full-scale toxicological assessment. Other uses involve occupational health considerations. Process intermediates may never reach the marketplace, but there will be certain individuals who are exposed to them and it will be important to know, from an occupational hazard standpoint, whether there is any risk to employees exposed to these intermediates.

Short-term tests identify potential for toxicity. Once potential has been established, the economics of development and use pattern of the compound will determine the subsequent steps in toxicological safety evaluation prior to marketing.

REFERENCES

1. AMES, B.N. 1979. Identifying environmental chemicals causing mutations and cancer. Science 204:587–593.
2. BRUSICK, D.J. 1979. Alterations of germ cells leading to mutagenesis and their detection. Environ. Health Perspect. 24:105–112.
3. BRUSICK, D.J. 1978. The role of short-term testing in carcinogen detection. Chemosphere 5:403–417.
4. BRUSICK, D.J. 1980. Principles of Genetic Toxicology. Plenum Press. New York.
5. BURNET, F.M. 1974. Intrinsic Mutagenesis: A Genetic Approach to Aging. Medical and Technical Publishing. Lancaster, England.
6. MCKUSICK, V.A. 1978. Mendelian Inheritance in Man: Catalogs of Autosomal Dominant, Autosomal Recessive and X-Linked Phenotypes. 5th edit. The Johns Hopkins University Press. Baltimore, Md.
7. BRUSICK, D.J., D.W. MATHESON & D.R. JAGANNATH. 1980. Commercial screening of environmental chemicals. *In* Chemical Mutagens: Principles and Methods for Their Detection. 6:81–107. Plenum Press. New York.
8. BRUSICK, D. 1981. Unified scoring system and activity definitions for results from *in vitro* and submammalian mutagenesis test batteries. *In* Health Risk Analysis. C.R. Richmond, P.J. Walsh & E. Copenhaver, Eds.:273–286. Proc. 3rd Life Sciences Symp. Gatlinburg, Tennessee. (October 27–30, 1980). Franklin Institute Press. Philadelphia.
9. MAGEE, P.N. 1977. The relationship between mutagenesis, carcinogenesis and teratogenesis. *In* Progress in Genetic Toxicology. D. Scott, B.A. Bridges & F.H. Sobels, Eds.:15–27. Elsevier/North-Holland Biomedical Press. Amsterdam.